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13. ABSTRACT *(Maximum 200 words)*
As exercise while wearing protective clothing exacerbates body heat storage, compared to exercise in the heat, and as exercise alters immune responses, it appeared interesting to examine immune and stress responses while wearing protective clothing during moderate exercise. Methods: Eight subjects completed two bouts of exercise at 45% VO2 max in a thermo neutral environment: once while wearing shorts only (Control trial, CON) and again while wearing protective clothing (PRO). Venous blood samples were taken to analyze TNF-a mRNA by RT-PCR in LPS simulated blood, plasma catecholamines, and cortisol. Blood cell count was analyzed by flow cytometry. Rectal temperature (Tre) was monitored continuously. Results: Exercise with PRO resulted in significantly greater increases in Tre (39.2 + 0.2°C in PRO versus 38.0 + 0.1°C in CON), plasma stress hormones (plasma epinephrine, norepinephrine, and cortisol increased by + 70%, 150% and 33%, respectively), and immunocompetent cell count (leukocyte, lymphocyte, T cytotoxic and NK cell counts were 14%, 18%, 35% and 37% higher, respectively), but the greater increase in T cytotoxic and NK cell counts was not significant. Only T helper lymphocyte count was lower (-43%). During both exercise trials, T helper lymphocytes were significantly decreased at the end of exercise and recovery. With or without protective clothing, exercise was associated with an inhibition of TNF-a expression in simulated monocytes (~50% at min 20 and 40, and ~30% at min 60). Discussion: Protective clothing wearing induces significant thermal challenge curing exercise. The inhibition of TNF-a appears to be mediated primarily by exercise and not the added thermal load associated with protective clothing.

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Immune Function During and After 60 min of Moderate Exercise Wearing Protective Clothing

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Introduction: As exercise while wearing protective clothing exacerbates body heat storage compared to exercise in the heat, and as exercise alters immune responses, it appeared worthwhile to examine immune and stress responses while wearing protective clothing during moderate exercise. ***Methods:*** Eight subjects completed two bouts of exercise at 45% $\dot{V}O_{2max}$ in a thermoneutral environment: once while wearing shorts only (Control trial, CON) and again while wearing protective clothing (PRO). Venous blood samples were taken to analyze TNF-α mRNA by RT-PCR in LPS stimulated blood, plasma catecholamines, and cortisol. Blood cell count was analyzed by flow cytometry. Rectal temperature (T_{re}) was monitored continuously. ***Results:*** Exercise with PRO resulted in significantly greater increases in T_{re} (39.2 ± 0.2°C in PRO vs. 38.0 ± 0.1°C in CON) and plasma epinephrine and norepinephrine (+70% and 150%, respectively). Plasma cortisol increased only at the end of PRO exercise (+33%). Leukocyte and lymphocyte cell count was 14% and 18% higher, respectively, but there were no significant changes in T cytotoxic and NK cell counts compared to the CON trial. Only T helper lymphocyte count was lower (−29%). During both exercise trials, T helper lymphocytes were significantly decreased at the end of exercise and recovery. With or without protective clothing, exercise was associated with an inhibition of TNF- α expression in stimulated monocytes (~ −50% at min 20 and 40, and ~ −30% at min 60). ***Discussion:*** Protective clothing wearing induces significant thermal challenge during exercise. The inhibition of TNF-α appears to be mediated primarily by exercise and not the added thermal load associated with protective clothing.

Keywords: exercise, hyperthermia, catecholamines, cortisol, leukocyte subsets, lymphocyte subsets, TNF- α mRNA.

SOLDIERS AND FIREFIGHTERS often work in environments which require them to wear protective clothing against chemical, biological, radiological, and nuclear agents. Wearing protective clothing for prolonged periods of time can impede exercise and metabolic heat loss, resulting in significant body heat storage (19). This body heat storage is exacerbated because protective clothing impedes body thermoregulation. Exercise while wearing protective clothing can cause noncompensable thermal stress (19), and for this reason, exercise duration is limited. It is well known that exercise alters immune responses, and some data suggest that heat stress exacerbates the effects observed during exercise of fixed duration in a thermoneutral environment. Furthermore, stress hormones and mobilization of immunocompetent cells during exercise-heat stress are increased compared to those observed during exercise in a thermoneutral environment (3,4). Alterations in lymphocyte subsets have also been shown to occur in response to moderate and prolonged exercise (18,23). However, to our knowledge, no studies have investigated the impact of wearing protective clothing during exercise on immune function. Specifically, the aim of this study was to examine the effect of protective clothing wearing (added thermal load and incapability to control body temperature) during moderate exercise on the circulating immunocompetent cell count and their functional response. To assess the functional response, we used a lipopolysaccharide (LPS) challenge test which has been shown to stimulate primarily monocytes (1) and result in an increased production of several cytokines. We examined TNF-α because it is one of the major proinflammatory cytokines. We hypothesized that the added thermal load associated with wearing protective clothing during exercise would lead to greater stress hormones that might decrease the TNF-α response to LPS stimulation.

METHODS

Subjects

Eight men volunteered to participate in the study. The volunteers were informed of the experimental procedures of the study and were allowed to withdraw from the study at any time. This investigation was approved by an appropriate Ethics committee (Committee for the Protection of Personnel in Biomedical Research, Grenoble, France) and all the volunteers provided written informed consent. Age, weight, and height (mean ± SEM) were

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24 ± 1 yr, 72 ± 2.1 kg, and 178 ± 2 cm, respectively. All of the volunteers were moderately active and familiar with treadmill exercise, and were nonsmokers. Their physical fitness was assessed by maximal O_2 uptake ($\dot{V}o_{2max}$) measured using a breath-by-breath automated gas exchange system (MedGraphics CPX/D, Medical Graphics Corporation, St Paul, MN) during a progressive treadmill test (Imbernon Jog-25, Lyon, France). Briefly, after a warm-up at 7 km · h^{-1}, the subjects ran at 11 km · h^{-1}, and the speed increased 1 km · h^{-1} every 3 min until exhaustion. The average $\dot{V}o_{2max}$ was 51.0 ± 1.4 ml · min^{-1} · kg^{-1}. The experiment took place in winter and spring.

Study Design

A complete crossover design was used in which each subject completed two exercise bouts, once while dressed in shorts only (Control trial, CON) and once while dressed in protective clothing (protection decontamination light clothes, PRO). At least 15 d separated the two trials, and both were conducted in the morning. The subjects were asked to refrain from strenuous exercise 2 d before each trial, to drink at least 2 L of water per day in order to maintain a euhydrated state, and to standardize their food consumption.

Each day, the volunteers reported to the laboratory at 0830 after a breakfast of standardized composition in carbohydrates, proteins, and lipids. Thereafter, the volunteers emptied their bladders and were fitted with a rectal probe. A thermistor (YSI 401 probe, Yellow Springs Instruments, Dayton, OH) inserted 10 cm beyond the external anal sphincter was connected to a portable recorder (HTM 8000, LE2IM, Lozanne, France). Rectal temperature (T_{re}) was measured continuously and recorded at 1-min intervals. They were weighed, and a polyethylene catheter (Angiocath 20GA 2in, Becton Dickinson, Sandy, UT) was inserted into an antecubital vein. Then the volunteers were dressed in shorts or protective clothing. They rested then for 30 min, standing on the treadmill, to stabilize hemodynamic readings. Prior to the start of exercise, a reference blood sample was taken. During both trials, the volunteers walked for 60 min in controlled thermal conditions (T_{db} = 25°C, RH = 35–45%, wind speed = 2 m · s^{-1}) at 6 km · h^{-1}. The slope of the treadmill was adjusted appropriately to ensure that each volunteer worked at 45% of their $\dot{V}o_{2max}$ on the basis of constant measurements of oxygen consumption during the trials. The slope was maintained for the last 5 min of exercise, and the speed was decreased to 4 km · h^{-1} for the avoidance of syncope at the end of exercise. At the end of the exercise, the subjects were undressed, emptied their bladders, and weighed. The exercise phase was followed by a recovery period which lasted 60 min in controlled thermal conditions (T_{db} = 25°C, RH = 35–45%) The subjects were dressed in bathrobes and seated in armchairs, except 30 min before the blood sample, to stabilize hemodynamic conditions.

Protective clothing: The subjects wore protective clothing against nuclear, biological, radiological, and chemical agents. The clothing used in this investigation is used by the French military and is manufactured by P. Boyé (protection decontamination light clothes, Sète, France). The external coat of the clothing is a proofing and the thermic isolation for this protective suit is 0.58 Clo. The protective suit eliminated the possibility for heat exchange with the external environment and did not allow evaporative sweat loss.

Blood sampling schedule: Blood samples (12 ml) were taken immediately prior to exercise, at minutes 20, 40, and 60 of exercise and after 60 min of recovery. The blood samples were collected into plastic syringes and transferred immediately to prechilled glass tubes containing specific anticoagulants for plasma.

Plasma volume measurements: Variations of plasma volume changes were calculated over time from hematocrit and hemoglobin concentration variations (performed on the Pentra 120 Retic; ABX-France, Montpellier, France) using the Dill and Costill equation (7). The hematocrit was multiplied by the factor (0.96*0.91) to correct for trapped plasma and to convert the venous hematocrit to whole body hematocrit, and the hemoglobin was multiplied by the factor 0.92 to convert the venous hemoglobin to whole body hemoglobin in accordance with Harrison et al. (12). Calculation of the Dill and Costill equation was used as reference points and was adapted to posture.

Hormonal analyses: After collection of blood into tubes containing lithium-heparin, samples were centrifuged at 3000 g for 10 min. The supernatant was removed and stored at −80°C until analysis. Plasma catecholamines were measured using high performance liquid chromatography with electrochemical detection. Plasma cortisol was assayed using commercial kits (ref TKC01, DPLC, LA Garenne Colombes, France).

Leukocyte count measurements: Blood (3 ml) was placed in EDTA tubes and analyzed for differential white cell counts as routinely performed on the Pentra 120 Retic (ABX-France).

Lymphocyte subpopulation analysis by flow cytometry: Blood (3 ml) was placed in EDTA tubes. A stock solution of 1 mmol · L^{-1} of chloromethyl X rosamine in dimethyl sulfoxide was diluted to 1:50 in dimethyl sulfoxide just before use. Of this intermediate concentration of chloromethyl X rosamine, 5 µl was added to $5*10^5$ blood cells, which were suspended in 1 ml of culture medium (RMPI-FCS). After incubation for 15 min at 37°C, cells were washed in saline phosphate buffer and stained with a fluorescein isothiocyanate-conjugated anti-CD3 mAb (clone G4.18, PharMingen, San Diego, CA). Cells were washed once, suspended in 500 µl saline phosphate buffer, and immediately analyzed by flow cytometry.

Monocyte stimulation and TNF-α mRNA quantification by real-time polymerase chain reaction (PCR): We suspended 50 µl of blood in 450 µl of culture medium (mercaptoethanol-RPMI-1640) supplemented with glutamax (Seromed Biochrom, ref: Merck Eurolab 55,172.01), and mercaptoethanol ($5*10^{-5}$ mol · L^{-1}), and stimulated with

LPS (Sigma-Aldrich, ref L6143), a component of the cell wall of Gram-negative bacteria at 1 $\mu g \cdot ml^{-1}$. Another aliquot of 50 µl was suspended in 450 µl of mercaptoethanol-RPMI-1640 without LPS for non-stimulated control. After incubating the cells for 2 h, they were spun, suspended in 200 µl of lysis buffer (MagNA Pure LC mRNA Isolation Kit, I-Lysis Buffer, ref: 3,246,744, Roche, Meylan, France), and stored at −20°C until TNF-α mRNA quantification. Total RNA was isolated using MagNA Pure LC mRNA Isolation Kit I (Roche). Reverse transcription of mRNA (22) was carried out in a 60-µl final volume using 300 U M-MLV reverse transcriptase (in vitrogen) according to manufacturer's instructions with 500 ng oligo$(dT)_{12\text{-}18}$ and 50 U ribonuclease inhibitor (RNAse out, Promega). PCR was carried out by Light Cycler fast start DNA master SYBR Green kit (Roche) using 0.5 µl of cDNA, corresponding to 30 ng of total RNA, in a 20-µl final volume, 3-4 $mmol \cdot L^{-1}$ $MgCl_2$, and 0.4 µM each primer (final concentration). Quantification PCR was performed using a Light Cycler (Roche) for 45 cycles at 95°C for 20 s, specific anneling temperature for 4–5 s, and 72°C for 8 s. Amplification specificity was checked using the melting curve according to the manufacturer's instructions.

Statistical analysis: Data analysis was performed with the Statistica® package (Statsoft Inc., Maisons-Alfort, France). Statistical differences were calculated with a two-way repeated-measures ANOVA design; when an overall difference was found, individual stages were compared with the Tukey post hoc test. Data are presented as mean ± SEM, and the null hypothesis was rejected when $P < 0.05$ for all analyses.

RESULTS

Changes in T_{re} (**Fig. 1**) increased significantly during 60 min of exercise without and with protective clothing with final T_{re} of 38.0 ± 0.05°C and 39.2 ± 0.15°C, respectively ($P < 0.01$ compared to CON; $P < 0.01$ compared to time 0). T_{re} decreased during the recovery, but remained higher during the PRO trial with 37.7 ± 0.05°C vs. 37.2 ± 0.07°C during the CON trial ($P < 0.01$ compared to time 0 and $P > 0.01$ compared to CON).

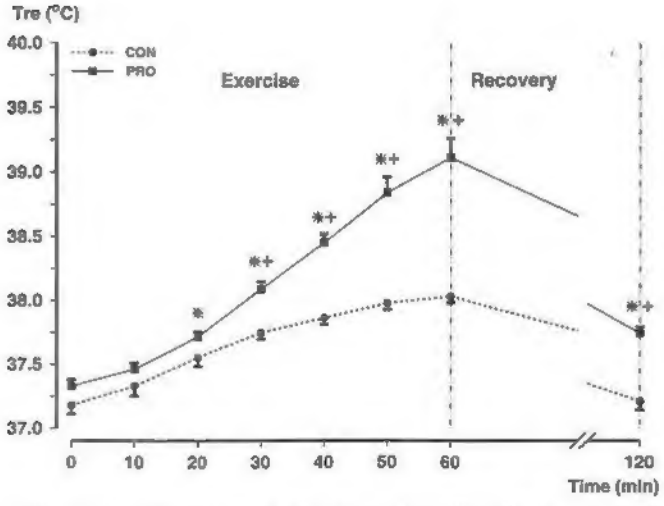

Fig. 1. Plot of T_{re} vs. time (°C) in the CON (black circles), and PRO (black squares) trials. Values are means ± SEM. † Significant difference from CON trial ($P < 0.01$). * Significant difference from time 0 ($P < 0.01$).

Plasma volume changes (**Table I**) did not change significantly during both exercise bouts. The values increased during the recovery ($P < 0.05$ compared to time 0). Values were corrected for plasma volume changes. The loss of weight was significantly higher during the PRO than during the CON trial (775 ± 38 g and 1318 ± 149 g, for CON and PRO trials, respectively, $P < 0.01$).

Plasma epinephrine levels (**Fig. 2**) were significantly increased only during the PRO trial (106 ± 10 $pg \cdot ml^{-1}$ and 152 ± 36 $pg \cdot ml^{-1}$ at 20, 40, and 60 min of exercise, respectively, $P < 0.01$ compared to CON; $P < 0.01$ compared to time 0). At time 60, plasma epinephrine showed a slight but significant decrease ($P < 0.05$ compared to time 40). Plasma norepinephrine levels (**Fig. 3**) increased significantly during the CON trial (571 ± 29 $pg \cdot ml^{-1}$, 627 ± 33 $pg \cdot ml^{-1}$, and 521 ± 31 $pg \cdot ml^{-1}$ at 20, 40, and 60 min of exercise, respectively) and during the PRO trial (937 ± 65 $pg \cdot ml^{-1}$, 1567 ± 191 $pg \cdot ml^{-1}$, and 1511 ± 184 $pg \cdot ml^{-1}$ at min 20 to 60).

Plasma cortisol (**Fig. 4**) decreased slightly and was not significantly different during the CON and PRO trials at min 40 (12 ± 1 $\mu g \cdot dl^{-1}$ and 10 ± 1 $\mu g \cdot dl^{-1}$ for the CON and PRO trials, respectively). Plasma cortisol increased significantly at the end of PRO exercise (16 ± 1 $\mu g \cdot dl^{-1}$, $P < 0.001$ compared to CON; $P < 0.001$ compared to time 60). Due to technical reasons, plasma epinephrine, norepinephrine, and cortisol could not be assessed in the blood samples collected during the recovery phase.

The exercise induced a significant increase of total leukocytes (Table I) in both trials from 20 min until the end of exercise and recovery. At the end of PRO exercise, the leukocyte cell counts were 14% higher than CON trial values ($P < 0.05$). This increase was observed for lymphocytes. With exception of neutrophil values ($P < 0.05$ compared to CON trial), all leukocyte counts were similar during the recovery.

T lymphocytes ($CD3^+$) (**Table II**) increased during both trials when compared to time 0, but were higher during PRO compared to the CON trial. T helper lymphocytes ($CD3+CD4^+$) (Table II) followed an opposite evolution of total T lymphocytes. They were clearly lowered at the end of CON and PRO exercise (40–50% of the cell count) ($P < 0.01$ compared to time 0) and recovery ($P < 0.001$ compared to time 0).

T cytotoxic lymphocyte ($CD3^+CD8+$) (Table II) changes were similar to total T lymphocytes changes. The greater increase during PRO was not significant when compared to the CON trials. NK cell ($CD16^+CD56+$) (Table II) count was significantly elevated during the CON and PRO exercise and the recovery phase. During the PRO recovery phase, NK cell count decreased 37% when compared to the end of exercise ($P < 0.05$). The ratio of T helper/T cytotoxic cells did not change significantly during either trial (Table II).

TABLE I. MEAN VALUES (± SEM) OF TOTAL LEUKOCYTE ($10^3 \cdot \mu l^{-1}$), NEUTROPHIL ($10^3 \cdot \mu l^{-1}$), LYMPHOCYTE ($10^3 \cdot \mu l^{-1}$), AND MONOCYTE ($10^3 \cdot \mu l^{-1}$) CHANGES CORRECTED FOR PLASMA VOLUME CHANGES.

			Exercise			Recovery
	Trial	0 min	20 min	40 min	60 min	120 min
Plasma volume (Δ%)	CON	0	+0.3 ± 1.2	+1.8 ± 1.7	+3.2 ± 1.4	+7.3 ± 3.0*
	PRO	0	+0.1 ± 0.6	−1.8 ± 0.6	−2.9 ± 0.4†	+8.8 ± 2.2*
Total leukocyte count ($10^3 \cdot \mu l^{-1}$)	CON	4.4 ± 0.4	5.9 ± 0.4*	6.1 ± 0.5*	5.8 ± 0.5*	7.1 ± 0.7*
	PRO	4.4 ± 0.4	6.3 ± 0.5*	6.9 ± 0.4*	6.6 ± 0.5*†	7.6 ± 0.5*
Neutrophil count ($10^3 \cdot \mu l^{-1}$)	CON	2.6 ± 0.3	3.2 ± 0.3	3.3 ± 0.4*	3.3 ± 0.4*	4.2 ± 0.6*
	PRO	2.6 ± 0.3	3.5 ± 0.4*	3.8 ± 0.4*	3.8 ± 0.4*	4.9 ± 0.4*†
Lymphocyte count ($10^3 \cdot \mu l^{-1}$)	CON	1.3 ± 0.1	1.9 ± 0.1*	2.0 ± 0.1*	1.8 ± 0.1*	2.2 ± 0.2*
	PRO	1.3 ± 0.1	2.1 ± 0.1*	2.3 ± 0.2*†	2.1 ± 0.1*††	2.0 ± 0.1*
Monocyte count ($10^3 \cdot \mu l^{-1}$)	CON	0.34 ± 0.1	0.52 ± 0.1*	0.59 ± 0.1*	0.48 ± 0.1*	0.56 ± 0.06*
	PRO	0.32 ± 0.1	0.60 ± 0.1*	0.59 ± 0.1*	0.54 ± 01*	0.52 ± 0.03*

† Significant difference from the CON trial ($P < 0.05$); †† significant difference from the CON trial ($P < 0.01$); * significant difference from time 0 ($P < 0.001$).

Controls without LPS stimulation produced negligible quantities of TNF-α mRNA. The evolution of TNF-α mRNA expression after LPS stimulation was similar during both exercise trials (**Fig. 5**). TNF-α mRNA expression decreased significantly at min 20 (−58% ± 1 and −51% ± 4 for CON and PRO, respectively, $P < 0.05$ compared to time 0) and 40 (−54% ± 7 and −48% ± 5 for CON and PRO, respectively, $P < 0.05$ compared to time 0) during both trials and was not different between CON and PRO trials. TNF-α mRNA expression decrease was less significant at the end of exercise (−40% ± 2 and −28% ± 10 for CON and PRO, respectively) and at the end of the recovery phase (−33% ± 6 and −24% ± 4 for CON and PRO, respectively, $P < 0.05$ compared to time 0).

DISCUSSION

The main findings of this study are that 1) moderate intensity exercise with protective clothing resulted in significantly greater increases in T_{re} and plasma catecholamines during whole exercise and in plasma cortisol only at the end of exercise; 2) exercise-induced increases in circulating leukocytes and subsets, and lymphocytes and subsets were higher with protective clothing; and 3) the exercise-associated decrease in TNF-α mRNA production by stimulated monocytes was not affected by the additional thermal load associated with wearing protective clothing.

We observed a major increase in T_{re} and a greater loss of weight during the PRO exercise trial. Protective clothing is thermally very challenging (11), which results in significant heat storage (19). This thermal load induced greater plasma catecholamines during PRO compared to the CON trial, which is consistent with previous data during exercise in the heat (3,4,25). The slight decrease in catecholamines observed at the end of exercise was related to the decreased intensity of exercise at this time. Plasma cortisol increased only at the end of the PRO trial. At work levels below 50% $\dot{V}O_{2max}$, the cortisol con-

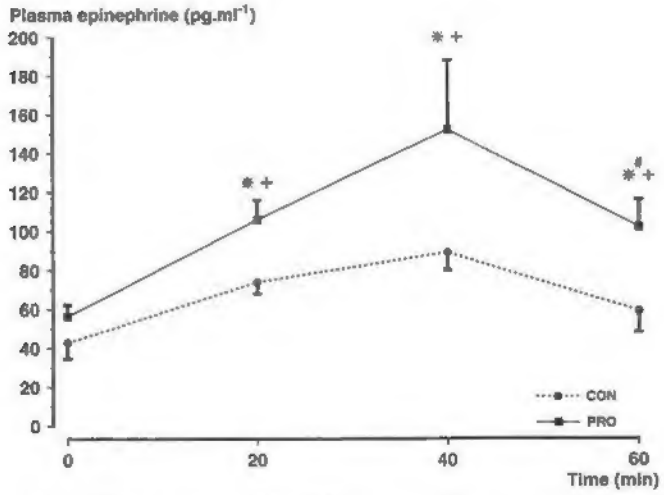

Fig. 2. Plot of plasma epinephrine (pg · ml^{-1}) vs. time in the CON (black circles) and PRO (black squares) trials, corrected for plasma volume changes. Values are means ± SEM. † Significant difference from the CON trial ($P < 0.01$). * Significant difference from time 0 ($P < 0.01$). # Significant difference from time 40 ($P < 0.05$).

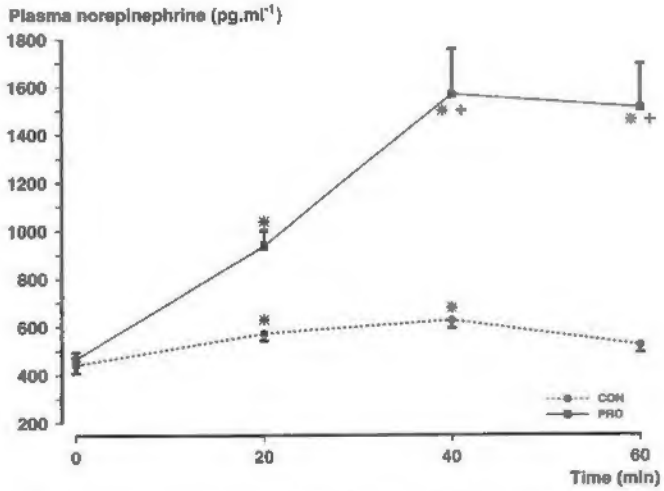

Fig. 3. Plot of plasma norepinephrine (pg · ml^{-1}) vs. time in the CON (black circles) and PRO (black squares) trials, corrected for plasma volume changes. Values are means ± SEM. † Significant difference from the CON trial ($P < 0.01$). * Significant difference from time 0 ($P < 0.01$).

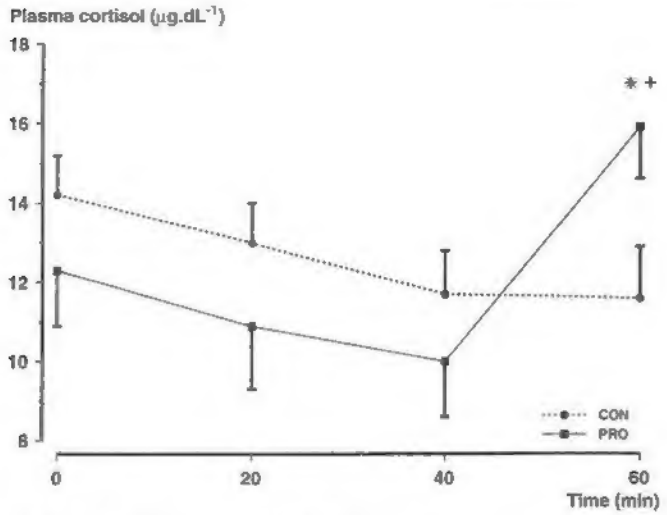

Fig. 4. Plot of plasma cortisol ($\mu g \cdot dl^{-1}$) vs. time in the CON (black circles) and PRO (black squares) trials, corrected for plasma volume changes. Values are means ± SEM. † Significant difference from the CON trial ($P < 0.01$). * Significant difference from time 0 ($P < 0.01$).

centration in plasma usually decreased, as in our study (8). The peak cortisol levels occurred after 40 min, which may mark the beginning of non-compensable thermal stress (19). In spite of the loss of weight, the low changes in plasma volume in both trials are consistent with previous data showing that plasma volume is preserved during and after moderate exercise (14).

Total Leukocyte and Subset Changes

Exercise with protective clothing lead to moderate leucocytosis, which has been shown previously to correlate with the intensity of exercise (15,20). This leucocytosis originates from an increase in lymphocyte and monocyte count. These responses are explained both by the rise in plasma catecholamines and the increase in blood flow (9). During the PRO trial, the increases in these leukocyte subsets were greater when compared to the CON trial, with an exception for monocytes. This phenomenon was previously observed during exercise-heat stress (4,5,10) and is believed to be related to increased catecholamine release (3,21,23).

Changes in Lymphocyte Subsets

During the CON exercise trial, we observed a significant increase in T cytotoxic lymphocytes and NK cells, whereas T helper lymphocytes decreased. Under similar conditions, Mitchell et al. (18) observed an increase in NK cell number and a slight reduction of T helper and T cytotoxic lymphocytes, while Kendall et al. (15) observed a transitory increase in T helper cells with no changes in T cytotoxic lymphocytes. According to Mac Ferlin et al. (17), the activity of NK cells could be increased after exercise. During the PRO trial, the increase in lymphocytes was significantly greater when compared to the CON trial. These results are consistent with observations during exercise-heat stress (10,18) and are related to greater increases in catecholamine release (3,21,23). Contrary to other immunocompetent cells, T helper cells decreased by approximately 50% during the PRO trial and this reduction persisted throughout the recovery period. Mitchell et al. (18) observed similar responses during exercise-heat stress. However, they also observed significant reductions in all the lymphocyte subsets. Furthermore, it has been shown that the T helper cells might decrease with intense exercise (15). As T helper cells decreased during the PRO trial and T cytotoxic cells increased in the same level, the ratio did not change significantly. Moderate exercise with an increased thermal stress may result in comparable immune responses as intense exercise.

TNF-α mRNA Changes After Monocyte Stimulation

Moderate exercise was associated with an inhibition of TNF-α expression in LPS-stimulated leukocytes. Starkie et al. (27–29) obtained similar results when TNF-α was examined with flow cytometry. They observed a marked decrease in the amount of TNF-α per cell after exercise. De Rjik et al. (6) observed a suppression of TNF-α production in the supernatant of whole blood incubated with LPS, while Rhind et al. (24) observed that,

TABLE II. MEAN VALUES (± SEM) OF LYMPHOCYTE ($10^3 \cdot ml^{-1}$), T HELPER LYMPHOCYTE ($10^3 \cdot \mu l^{-1}$), T CYTOTOXIC LYMPHOCYTE ($10^3 \cdot \mu l^{-1}$), AND NK CELL ($10^3 \cdot \mu l^{-1}$) CHANGES, CORRECTED FOR PLASMA VOLUME CHANGES.

		Exercise		Recovery
	Trial	0 min	60 min	120 min
T lymphocytes $CD3^+$ ($10^3 \cdot \mu l^{-1}$)	CON	0.95 ± 0.09	1.17 ± 0.07*	1.42 ± 0.07*
	PRO	0.93 ± 0.09	1.38 ± 0.13*†	1.36 ± 0.12*
T helper lymphocytes $CD3^+CD4+$ ($10^3 \cdot \mu l^{-1}$)	CON	0.49 ± 0.06	0.34 ± 0.03*	0.32 ± 0.03*
	PRO	0.48 ± 0.04	0.24 ± 0.02*	0.37 ± 0.04*
T cytotoxic lymphocytes $CD3^+CD8+$ ($10^3 \cdot \mu l^{-1}$)	CON	0.25 ± 0.02	0.31 ± 0.03*	0.38 ± 0.02
	PRO	0.26 ± 0.04	0.42 ± 0.10*	0.39 ± 0.06
NK cells $CD16^+CD56+$ ($10^3 \cdot \mu l^{-1}$)	CON	0.18 ± 0.03	0.35 ± 0.05*	0.38 ± 0.08*
	PRO	0.17 ± 0.02	0.48 ± 0.09*	0.30 ± 0.05*#
T helper/T cytotoxic ratio	CON	1.6 ± 0.1	1.5 ± 0.2	1.6 ± 0.1
	PRO	1.7 ± 0.2	1.3 ± 0.2	1.6 ± 0.2

† Significant difference from the CON trial ($P < 0.05$); * significant difference from time 0 ($P < 0.01$); # significant difference from the end of exercise ($P < 0.05$).

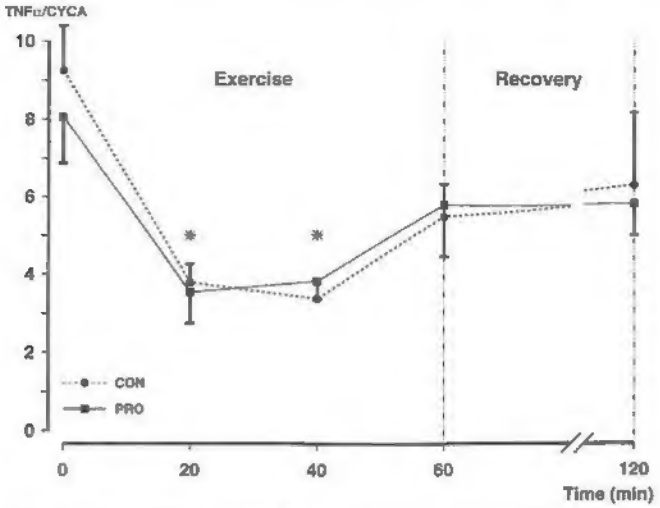

Fig. 5. Plot of TNF-α transcription in monocytes after LPS stimulation vs. time in the CON (black circles) and PRO (black squares) trials. Values are means ± SEM. * Significant difference from time 0 ($P < 0.01$).

during moderate exercise, human peripheral blood monocytes expressed increased levels of TNF-α. Elevated cortisol levels have been shown to play a major role in the inhibition of TNF-α secretion (6). The mechanism appears to act through the glucocorticoid receptors, which are intracellular receptors that exert immunosuppressive effects after agonist-induced translocation to the nucleus (13). TNF-α expression may be very sensitive to elevated glucocorticoid secretion (6). Catecholamines could also act on the reduced production of TNF-α mRNA by monocytes (2). In our study, TNF-α mRNA and plasma cortisol both decreased. The inhibition of TNF-α mRNA expression was similar during both CON and PRO trials. Starkie et al. (29) made the same observation during strenuous exercise in the heat, with similar T_{re} responses. They showed that heat had no effect on spontaneous intracellular cytokine production. However, in vitro studies show an effect of hyperthermia, catecholamines, and cortisol. An incubation of macrophages at 39.5°C decreased the transcription of TNF-α (26). In addition, other studies in vitro have shown that epinephrine added to whole blood while incubated with LPS decreased cytokine production (30). However, in our study, TNF-α mRNA exhibited similar changes during both the CON and PRO trials, whereas plasma catecholamines, plasma cortisol, and T_{re} had different responses. Yet these data are not sufficient to fully explain the reduction in TNF-α mRNA and the subsequent increase. The LPS stimulation test evaluates the functional response of the cells ex vivo. There is a cell mobilization during exercise and perhaps the cells taken at the end of exercise do not have the same origin. Lancaster et al. (16) have shown that exercise causes suppression of both expression and function of toll-like receptors on CD14+ monocytes. These receptors are implicated in the response to LPS. Moreover, exercise results in differential mobilization of two monocytes subpopulations with different toll-like receptor expressions (16). This could have an impact on the production of TNF-α.

In conclusion, moderate exercise while wearing protective clothing results in uncompensable heat stress, which is associated with elevated body temperature and increased plasma catecholamines and cortisol concentrations compared to control exercise. Increased plasma catecholamine concentration during the PRO exercise trial may be involved in the observed immunocompetent cell changes compared to control exercise. Plasma catecholamines, plasma cortisol, and T_{re} changes are not sufficient to fully explain the reduction in TNF-α mRNA after exercise.

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